Acquired Ras mutation and cancer survival

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***Short Abstract* —Ras point mutants are commonly found in cancer. Evidence suggests the specific mutant acquired may influence patient outcomes such as survival rates. We use our previously developed mathematical model of the Ras signaling network to investigate two of the more common Ras mutants. The model predicts that the mutant associated with the worse overall clinical survival produces a higher level of Ras pathway signaling. We confirm this prediction experimentally. This work suggests that observed clinical outcomes may in part depend upon differences in signal intensity that can be predicted with a mechanistic model of the cell signaling network.**

I. Introduction

RAS missense point mutations that result in increased total cellular RasGTP are commonly found in cancer and are believed to play a contributory role in cancer development [1]. Many different missense mutants have been found in human cancers; most commonly they occur at codon 12, 13, or 61. Studies have found that cancers with a glycine to valine mutation at codon 12 (RasG12V) tend to be more likely to result in death than other commonly acquired missense mutations, such as glycine to aspartate at codon 12 (RasG12D) [2, 3]. It has been hypothesized that this could be due to differences in the intensity of the RasGTP signal that results from the expression of each mutant [4]. Experimental evidence of different levels of RasGTP signal intensity for similar expression levels of different Ras mutants in similar cell types, however, is lacking. It has also been speculated that differences in intensity could be related to in vitro measurements of reaction rate constants for the different mutants [4]. However, the complex regulation of intracellular RasGTP levels can result in non-intuitive patterns of Ras pathway activation due to the same rate constants [5]. A more mathematically sophisticated model-based approach may therefore be of value.

II. Methods and Results

We use our previously described model of the Ras signaling network to investigate the intensity of the Ras pathway signal that results from RasG12V and RasG12D expression [5]. This model includes Ras and the different classes of signaling proteins that regulate cellular levels of RasGTP. Representative rate constants for all of these reactions were found in the published literature. Deviations from these values have previously been measured and published for RasG12V and RasG12D. Model simulations of the network including wild-type and mutant Ras protein predict that a greater increase in Ras pathway signal should occur for RasG12V expression than for RasG12D expression. This is true for low and high expression levels of these two mutants.

To investigate this prediction experimentally, RasG12V and RasG12D were expressed in cell culture. Flow cytometry was used to measure the amount of mutant Ras protein expressed and the amount of Ras pathway signal on a single-cell basis as described previously [5]. Observed patterns of Ras pathway matched well with model predictions. RasG12V caused a greater level of Ras pathway signal than RasG12D across a wide range of mutant protein expression levels.

III. Conclusion

This work combines computational analysis and traditional experimental methods to investigate the relationship between the biochemical properties of acquired oncogenic Ras point mutants, the intensity of Ras pathway signal in a cellular context, and the clinical outcome of an individual with a cancer containing a Ras point mutant. It suggests that observed differences in clinical outcome attributed to the specific acquired Ras missense mutant may reflect differences in the amount of Ras pathway signal produced by each. It also appears possible to predict these differences in Ras pathway signal using the rate constants for the biochemical reactions of each mutant.

References

[1] Malumbres M, Barbacid M. (2003) RAS oncogenes: the first 30 years. Nat Rev Cancer, 3(6): 459-65.

[2] Andreyev HJ, et al. (2001) Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. Br J Cancer, 85(5):692-6.

[3] Andreyev HJ, et al. (1998) Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. J Natl Cancer Inst, 90(9): 675-84.

[4] Al-Mulla F, Milner-White EJ, Going JJ, Birnie GD. (1999) Structural differences between valine-12 and aspartate-12 Ras proteins may modify carcinoma aggression. J Pathol, 187(4):433-8.

[5] Stites EC, Trampont PC, Ma Z, Ravichandran KS (2007) Network analysis of oncogenic Ras activation in cancer. Science, 318(5849): 463-7.

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